
PHYSICO-CHEMICAL QUALITY, BOTANICAL ORIGIN AND ANTIOXIDANT PROPERTIES OF FLORAL HONEYS FROM CAMPOS GERAIS REGION, BRAZIL

Débora Maria Borsato, Paulo Vitor Farago, Cynthia Fernandes Pinto da Luz, Severino Matias De Alencar and Mareci Mendes De Almeida

SUMMARY

The aim of this study was to analyze the physicochemical quality, botanical origin and antioxidant activity of Brazilian commercial honeys labeled as monofloral. Thirteen honey samples of Africanized honey bee (*Apis mellifera* L.) were provided by beekeepers from Campos Gerais region of Paraná State, Southern Brazil. Most of the samples ($n= 8$) showed physicochemical results in accordance with the International and Bra-

zilian legal requirements. Regarding pollen analysis, samples were classified as unifloral (5), bifloral (3) and heterofloral (5) honeys. For some honey samples (5), divergence between the label data and the results of melissopalynological analysis was observed. A high correlation coefficient ($r= 0.8379$) between total phenolic content and antioxidant activity was observed.

CALIDAD FÍSICO-QUÍMICA, ORIGEN BOTÁNICO Y PROPIEDADES ANTIOXIDANTES DE MIELES FLORALES DE LA REGIÓN DE CAMPOS GERAIS, BRASIL

Débora Maria Borsato, Paulo Vitor Farago, Cynthia Fernandes Pinto da Luz, Severino Matias De Alencar y Mareci Mendes De Almeida

RESUMEN

El objetivo de este estudio fue analizar las propiedades físico-químicas, el origen botánico y la actividad antioxidante de mieles comerciales de Brasil rotuladas como monoflorales. Trece muestras de miel de abeja (*Apis mellifera* L.) fueron cedidas por los apicultores de la región de Campos Gerais del Estado de Paraná, sur de Brasil. La mayoría de las muestras ($n= 8$) mostraron resultados físico-químicos de acuerdo con los

requisitos legales internacionales y brasileños. En cuanto al análisis del polen, las muestras fueron clasificadas como mieles monoflorales (5), biflorales (3) y heteroflorales (5). Para algunas muestras de miel (5) hubo divergencia entre los datos del rótulo y los resultados del análisis palinológico de las mieles. Se observó un alto coeficiente de correlación ($r= 0,8379$) entre el contenido de fenoles totales y la actividad antioxidante.

Introduction

The *Codex Alimentarius* Commission (Codex, 2001) defines honey as a “natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants,

which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature”.

Honey is a very traditional food and is used by many consumers due to its particular nutritional quality, high energy values, medicinal

uses (as antioxidant and anti-septic) and sensorial features (Zumla and Lulat, 1989). Moreover, some aspects of the commercially available honeys, such as price and quality, are usually related to their botanical origin or chemical composition.

Floral honeys can be classified according to their nectar

and pollen contents. For monofloral honeys, nectar is obtained mainly from a single botanical species. Otherwise, multifloral samples result from nectars of several botanical taxa. Particular interest about these hive products is due to their remarkable flavor and higher sale price. To determine the floral origin of honeys, melissopal-

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Débora Maria Borsato. Pharmacist and M.S. in Food Science and Technology, Universidade Estadual de Ponta Grossa (UEPG), Brazil. Ph.D. in Pharmaceutical Sciences, Universidade Federal de Paraná (UFPR), Brazil. Postdoctoral Fellow, UEPG, Brazil. e-mail: dmborsato@yahoo.com.br

Paulo Vitor Farago. Pharmacist, UEPG, Brazil. M.S. in Biologi-

cal Sciences (Botany) and Ph.D. in Chemistry, UFPR, Brazil. Professor, UEPG, Brazil. Address: Departamento de Ciências Farmacêuticas, UEPG. Av. Carlos Cavalcanti, 4748. CEP 84030-900 Brasil. e-mail: pvfarago@uepg.br

Cynthia Fernandes Pinto da Luz. Biologist, Universidade de Santa Úrsula, Brazil. M.S. and Ph.D. in Geology, Federal Uni-

versity of Rio de Janeiro, Brazil. Researcher, Instituto de Botânica, Brazil. e-mail: cyluz@yahoo.com.br

Severino Matias De Alencar. Agronomist and M.S. in Agronomy, Universidade Federal Rural do Rio de Janeiro, Brazil. Ph.D. in Food Science, Universidade Estadual de Campinas, (UNICAMP), Brazil. Professor, Escola Superior de Agricultura

“Luiz de Queiroz” Universidade de São Paulo, Brazil. e-mail: alencar@esalq.usp.br

Mareci Mendes De Almeida. Pharmacist, UEPG, Brazil. M.S. in Sciences (Biochemistry), UFPR, Brazil. Ph.D. in Food Science, UNICAMP, Brazil. Professor, UEPG, Brazil. e-mail: mareci@uepg.br

QUALIDADE FÍSICO-QUÍMICA, ORIGEM BOTÂNICA E PROPRIEDADES ANTIOXIDANTES DE MÊIS FLORAIS DA REGIÃO DOS CAMPOS GERAIS, BRASIL

Débora Maria Borsato, Paulo Vitor Farago, Cynthia Fernandes Pinto da Luz, Severino Matias De Alencar e Mareci Mendes De Almeida

RESUMO

O objetivo deste estudo foi investigar a qualidade físico-química, a origem botânica e a atividade antioxidante de méis comerciais brasileiros rotulados como monoflorais. Treze amostras de mel de abelha (*Apis mellifera* L.) foram cedidas por apicultores da região dos Campos Gerais do Paraná, Sul do Brasil. A maioria das amostras ($n=8$) apresentou parâmetros físico-químicos em conformidade com as exigências legais in-

ternacionais e brasileiras. Quanto à análise polínica, as amostras foram classificadas como méis monoflorais (5), biflorais (3) e heteroflorais (5). Para algumas amostras de mel (5), houve divergência entre os dados de rotulagem e os resultados da análise melissopalínológica. Um alto coeficiente de correlação ($r=0,8379$) entre o teor de fenólicos totais e a atividade antioxidante foi observado.

inology has been widely used because it is a quite simple and low cost method (Moreira and De Maria, 2001; Barth, 2004; Von Der Ohe *et al.*, 2004).

Some papers have focused on the determination of antioxidant activity from bee (*Apis mellifera* L.) honey (Giorgi *et al.*, 2011; Hussein *et al.*, 2011; Kishore *et al.*, 2011; Liberato *et al.*, 2011). Antioxidant properties of honeys can be related to enzymatic and non-enzymatic components (National Honey Board, 2011) which depend mainly upon the floral source of the honeys (Gheldof and Engeseth, 2002; Nagai *et al.*, 2006). Besides the relevance of antioxidants for quality control of bee honeys, these compounds have also been shown to have biological effects that can provide antibacterial, anti-inflammatory, anti-allergy, and anti-thrombotic activities (Cook and Sammon, 1996).

Campos Gerais, a region of Southern Brazil, can be considered an important supplier of floral honeys due to their high production value and increased exports. However, there is a lack of data about chemical and biological properties of floral honeys from this region, which are required to enhance the commercial opportunities for beekeepers and to provide knowledge about the potential medicinal use of these particular honeys. Therefore, the aim of this paper was to analyze the physicochemical quality, botanical origin and antioxidant activity of floral honeys from the Campos Gerais region.

Materials and Methods

Samples

Thirteen honey samples of Africanized honey bee (*A. mellifera*) were kindly provided by beekeepers from the Campos Gerais region, Paraná state, Southern Brazil. The samples commercially marketed as monofloral honeys were three from *Eucalyptus* (Myrtaceae), two from *Baccharis* (Asteraceae), two from *Croton* (Euphorbiaceae), one from *Citrus* (Rutaceae), one from *Piptadenia* (Fabaceae), one from *Mimosa* (Fabaceae), one from *Lithraea* (Anacardiaceae), one from *Vernonia* (Asteraceae), and one from *Eryngium* (Apiaceae). Honey samples were collected in 2007, and immediately cooled at $4 \pm 2^\circ\text{C}$ for physicochemical analyses.

Physicochemical analyses

Physicochemical analyses were performed in order to determine the compliance of the honey samples to national

and international requirements. Moisture, reducing sugars, sucrose, ash, free acidity, pH, diastase activity, hydroxymethylfurfural (HMF) content and Pfund color were measured in triplicate for the honey samples, according to the current standard methods (Table I) proposed in the literature (Bianchi, 1981; Komatsu, 1996; Bogdanov *et al.*, 1997; AOAC, 2000; Santos *et al.*, 2003; Vargas, 2006; Borsato *et al.*, 2010).

Concerning the Kirkwood equation (Kirkwood *et al.*, 1960, 1961), samples were discriminated in floral honeys or honeydew honeys according to the index resulting from the relationship among pH, ash and reducing sugars. If applicable, values of mean and standard deviation (SD) were calculated for the achieved experimental data.

Pollen analysis

To confirm the monofloral origin of the samples, pollen analysis was carried out using

the procedure recommended by the International Commission for Bee Botany (Louveau *et al.*, 1970) and Barth (1989). Briefly, a sample of 10g of crude honey was dissolved in 20ml of distilled water, centrifuged at 2000rpm during 10min, washed once with 10ml of distilled water, centrifuged again and part of the sediment embedded in glycerin jelly without previous acetolysis. The pollen sediment was transferred to 75x25mm slides; after being warmed slightly, the melted jelly with pollen sediment was covered by a 22mm cover glass and the latter sealed with paraffin wax (Nair 1960). Pollen grains were identified with the help of standard slides prepared from the local flora and descriptions of pollen grains obtained from the specialized literature (Barth, 1989; Takeda *et al.*, 2000, 2001, 2002a, 2002b; Melhem *et al.*, 2003). Quantitative analysis was performed by consecutively counting 100 pollen grains per replicate per sample. The pollen grains were classed into one of the follow-

TABLE I
PHYSICOCHEMICAL ANALYSES PERFORMED IN HONEY SAMPLES OF AFRICANIZED HONEY BEE (*Apis mellifera* L.) FROM CAMPOS GERAIS REGION OF BRAZIL

Physicochemical analyses	Method	Reference
Moisture	Refractometry	AOAC, 2000
Reducing sugars	Lane-Eynon titration	Bogdanov <i>et al.</i> , 1997
Sucrose	Lane-Eynon titration	Bogdanov <i>et al.</i> , 1997
Ash	Gravimetry	Borsato <i>et al.</i> , 2010; Vargas, 2006
Free acidity	Direct titration	Komatsu, 1996; Bogdanov <i>et al.</i> , 1997
pH	Potentiometry	Komatsu, 1996; Bogdanov <i>et al.</i> , 1997
Activity of diastase	Spectrophotometry	Santos <i>et al.</i> , 2003
Hydroxymethylfurfural content	Spectrophotometry	AOAC, 2000
Pfund color	Spectrophotometry	Bianchi, 1981

ing four pollen frequency classes: dominant (>45%), accessory (15-45%), important isolated (3-15%) and occasional isolated (<3%) pollen types. In order to evaluate the botanical origin of the honeys, records of the presence of pollen grains from nectariferous and non-nectariferous plants (entomophilous and anemophilous ones) were also performed according to the literature (Barth, 1989, 2005).

Determination of total phenolic contents

Extraction was performed according to Ferreres *et al.* (1991) with some modifications. A mass of 80g of honey samples were thoroughly mixed with five parts (400ml) of distilled water and adjusted to pH 2 with concentrated HCl, until becoming completely fluid by stirring with a magnetic stirrer at room temperature. The fluid samples were then filtered through cotton wool to remove solid particles. The filtrate was mixed with 80g Amberlite XAD-2 (pore size 9nm, particles size 0.3-1.2mm) and stirred with a magnetic stirrer for 15min. Afterwards, the Amberlite particles were packed in a glass column (25x2cm) and the column washed with 100ml of acidified water (pH 2, with HCl) and subsequently rinsed with 300ml of distilled water to remove all sugars and other polar constituents of honey. The phenolic compounds remained absorbed on the column and were eluted with 300ml of methanol. The methanol extract was concentrated to almost dryness under reduced pressure in a rotary evaporator at 60°C. The residue was re-suspended in methanol to a volume of 10ml.

The concentration of total phenolic contents in the obtained phenolic fractions was determined by the Folin-Ciocalteu procedure (Singleton *et al.*, 1999). Each phenolic fraction (0,5ml) was mixed with 2.5ml of 0.2mol·l⁻¹ Folin-Ciocalteu reagent for 5min and, then, 2ml of 4% Na₂CO₃ was added. After incubation at room temperature for 2h in the dark, the absor-

TABLE II
PHYSICOCHEMICAL RESULTS FOR HONEY SAMPLES OF AFRICANIZED HONEY BEE (*Apis mellifera* L.) FROM CAMPOS GERAIS REGION OF BRAZIL

Physicochemical parameters	Reference values *		Mean	SD	Range
	Floral honey	Honeydew honey			
Moisture (%)	≤20	≤20	17.39	1.12	15.10-18.43
Reducing sugars (%)	≥65	≥60	72.02	1.44	69.97-74.63
Sucrose (%)	≤6	≤15	3.24	1.12	1.25-5.07
Ash (%)	≤0.6	≤1.2	0.27	0.16	<i>n.d.</i> -0.52
Acidity (mEq·kg ⁻¹)	≤50	≤50	16.73	7.23	8.75-37.92
Ph	<i>n.e.</i>	<i>n.e.</i>	4.43	0.30	3.91-4.91
Activity of diastase (Göthe) **	≥8	≥8	5.63	3.66	1.70-13.57
Hydroxymethylfurfural (mg·kg ⁻¹)	≤60	≤60	3.00	4.13	<i>n.d.</i> -14.47
Color (mm Pfund)	Water-white to dark-amber	Water-white to dark-amber	Light amber (66.99)	<i>n.a.</i>	Water-white to dark amber (4.38-45.51)

* According to the Brazilian requirements (Brasil, 2000).

** If HMF<15mg·kg⁻¹, the official lower limit for diastase is ≥3 Göthe.

n.a.= not applicable, *n.d.*= not detected, *n.e.*= not established.

bance of the reaction mixture was measured at 740nm against a methanol blank. The total phenolic content was expressed in mg per 100g of honey as catechin equivalent (CE).

Antioxidant activity

The scavenging activity of honey samples for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described in Yen and Wu (1999) and Chen *et al.* (2003). Briefly, the extracted phenolic fractions from honey samples were previously diluted to final concentration of 10% in methanol. A volume of 300µl of a 0.5mmol·l⁻¹ DPPH-ethanol solution was added to 3ml of ethanol and 500µl of sample dilution. The samples were kept at room temperature in the dark. After 40min, the absorbance values were measured at 517nm and converted into the percentage antioxidant activity by Eq. 1:

Antioxidant activity (%) =

$$100 - \left[\left(\frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}} \right) \times 100 \right] \quad (1)$$

where A: absorbance value.

Ethanol (3,3ml) plus 500µl of each previously diluted phenolic fraction was used as blank, a DPPH solution (300µl; 0.5mmol·l⁻¹) plus ethanol

(3,5ml) was used as a negative control, and an ethanol solution of α-tocoferol (200mg·l⁻¹) was used as a positive control. The correlation coefficient to determine the relationship between total phenolic content and antioxidant activity was calculated using MS Excel® software (CORREL statistical function).

Results and Discussion

Physicochemical analyses

The results obtained in the physicochemical analyses of honey samples of Africanized honey bee (*A. mellifera*) from Campos Gerais region, Southern Brazil, are shown in Table II.

All samples showed values of moisture, reducing sugars, sucrose, ash, free acidity, hydroxymethylfurfural and Pfund color in agreement with the requirements of the Brazilian legislation (Brasil, 2000), the *Codex Alimentarius* Commission (Codex, 2001) and the European Honey Commission (Bogdanov *et al.*, 1997).

Analytical data for pH resulted in values from 3.91 to 4.91. Legal documents from international and national institutions have no reports about pH for bee honeys (Bogdanov *et al.*, 1997; Brasil, 2000). According to Crane (1983), changes in pH can be due to floristic composition and floral diversity of the region. Moreover, a singular soil composition and taxa association can

influence the final honey composition and pH.

Values ranging from 1.70 to 13.57 were obtained for diastase on the Göthe scale. Regarding the standards indicated by the Brazilian legislation (Brasil, 2000), a minimum diastase index of 8 is required in this scale. However, a minimum of 3 is also allowed for diastase if the HMF content is within the limit of 15mg·kg⁻¹. Five honey samples (numbered 4, 5, 8, 12 and 13) showed a diastase index <3. Diastase is a thermolabile α-amylase responsible for splitting starch chains into dextrans and maltose (White, 1975), and was initially used as a possible means of distinguishing between natural and artificial honeys. However, diastase is also widely recognized as a parameter for the evaluation of honey freshness, because its activity decreases in old or heated honeys (White, 1975; Bogdanov *et al.*, 1997; Tosi *et al.*, 2008). Commercial syrups obtained from totally or partially-inverted sucrose by acid hydrolysis and heating of sugar cane can reduce the diastase value of honeys. High-fructose syrups prepared from corn starch by enzymatic activities of amylases and isomerases show diastase in a normal range, but reveal a high HMF content. Some of these changes can be related to the results verified for the five honeys samples that exceeded the reported limits.

A total of nine bee honey samples (71.43%) were classified as light-amber and extra light-amber. Bee honeys with light colors usually show higher commercial values than dark ones. Also, the color of bee honeys is a wide-ranging property because some chemical features as fructose/glucose proportion, instability of fructose in acid medium, nitrogen content and presence of free amino acids, phenolic compounds and ash have a remarkable influence on browning (Bath and Sing, 1999).

A. mellifera extracts nectar from flowers to produce floral honeys. However, when bees collect sweet fluids that exude from botanical species usually visited by plant-sucking insects, honeydew honeys are obtained (Brasil, 2000). The differentiation between floral honey and honeydew or mixed honey was performed by a linear discriminant function (Eq. 2) as proposed by Kirkwood *et al.* (1960, 1961). For the evaluated samples, all bee honeys showed X values >73.1 and were considered as floral products.

$$X = -8.3x_1 - 12.3x_2 + 1.4x_3 \quad (2)$$

where x_1 : pH, x_2 : honey ash content in percentage, and x_3 : percentage of reducing sugars

Pollen analysis

A total of 53 pollen types were observed in the pollen spectra of the evaluated samples. In spite of their high frequency in some samples, the polliniferous types *Cecropia* (Moraceae) and *Piper* (Piperaceae) were removed from the total due to the fact that they do not contain nectar. For the same reason, all anemophilous types, e.g. *Amaranthus*, Cyperaceae, *Celtis*, Poaceae and *Trema*, were also excluded. However, anemophilous pollens can indicate the geographic origin of the samples. A total of ten nectariferous pollen types were considered as dominant or accessory, according to their frequency: *Citrus* (Rutaceae), *Eucalyptus* (Myrtaceae), *Ilex* (Aquifoliaceae), *Lithraea* (Ana-

TABLE III
BOTANICAL ORIGIN FOR HONEY SAMPLES OF AFRICANIZED HONEY BEE (*Apis mellifera* L.) FROM CAMPOS GERAIS REGION OF BRAZIL

Samples	Botanical origin declared by the beekeeper/label	Botanical origin determined by pollen analysis
1	eucalipto (<i>Eucalyptus</i> sp.)	eucalipto (<i>Eucalyptus</i> sp.)
2	bracatinga (<i>Mimosa scabrella</i>)	bracatinga (<i>Mimosa scabrella</i>)
3	aroeira (<i>Lithraea</i> sp.)	pau-de-malho (<i>Machaerium</i> sp.), yerba mate (<i>Ilex</i> sp.) and aroeira (<i>Lithraea</i> sp.)
4	assa-peixe (<i>Vernonia</i> sp.)	cipó-uva (<i>Serjania</i> sp.), eucalipto (<i>Eucalyptus</i> sp.), orange (<i>Citrus</i> sp.) and assa-peixe (<i>Vernonia</i> sp.)
5	caraguatá (<i>Eryngium</i> sp.)	angico (<i>Piptadenia</i> sp.)
6	capixingui (<i>Croton floribundus</i>)	eucalipto (<i>Eucalyptus</i> sp.) and laranjinha-do-mato (<i>Zanthoxylum</i> sp.)
7	eucalipto (<i>Eucalyptus</i> sp.)	eucalipto (<i>Eucalyptus</i> sp.)
8	orange (<i>Citrus</i> sp.)	orange (<i>Citrus</i> sp.)
9	eucalipto (<i>Eucalyptus</i> sp.)	yerba mate (<i>Ilex</i> sp.) and aroeira (<i>Lithraea</i> sp.)
10	vassoura (<i>Bacharis</i> sp.)	laranjinha-do-mato (<i>Zanthoxylum</i> sp.), yerba mate (<i>Ilex</i> sp.) and aroeira (<i>Lithraea</i> sp.)
11	vassoura (<i>Bacharis</i> sp.)	aroeira (<i>Lithraea</i> sp.), angico (<i>Piptadenia</i> sp.) and vassoura (<i>Bacharis</i> sp.)
12	angico (<i>Piptadenia</i> sp.)	angico (<i>Piptadenia</i> sp.) and <i>Myrcia</i> type
13	capixingui (<i>Croton floribundus</i>)	laranjinha-do-mato (<i>Zanthoxylum</i> sp.), yerba mate (<i>Ilex</i> sp.) and <i>Myrcia</i> type

cardiaceae), *Machaerium* (Fabaceae), *Mimosa scabrella* (Fabaceae), *Myrcia* (Myrtaceae), *Piptadenia* (Fabaceae), *Serjania* (Sapindaceae) and *Zanthoxylum* (Rutaceae). No honeydew elements such as microalgae, fungal mycelia, and spores were verified in the studied samples.

Considering pollen spectra, five samples were identified as monofloral honeys: two honeys of 'eucalipto' (*Eucalyptus* type, Myrtaceae), one sample of orange (*Citrus* type, Rutaceae), one honey of 'angico' (*Piptadenia* type, Fabaceae), and one sample of 'bracatinga' (*Mimosa scabrella* type, Fabaceae). Three samples were classified as bifloral honey: one of 'eucalipto' (*Eucalyptus* type, Myrtaceae) and 'laranjinha-do-mato' (*Zanthoxylum* type, Rutaceae), one of 'yerba mate' (*Ilex* type, Aquifoliaceae) and 'aroeira' (*Lithraea* type, Anacardiaceae), and one of 'angico' (*Piptadenia* type, Fabaceae) and *Myrcia* type, Myrtaceae. Other five samples were classified as polyfloral honeys (Table III).

Regarding the five honey samples that were considered monofloral, four samples (1, 2, 7 and 8) confirmed the botanical origin as reported by beekeepers. Also, other samples (3, 4, 11 and 12)

were partially composed by the botanical origin indicated by beekeepers. On the other hand, no contribution of the declared species was observed in honeys numbered 5, 6, 9, 10 and 13. These data demonstrate that there is some divergence between the botanical origin mentioned by beekeepers and the results of melissopalynological analysis, as also observed by Horn (1997) and Barth *et al.* (2005).

Price and quality of honeys are usually related to their floral origin and chemical composition. Particularly in the region of Campos Gerais and due to their remarkable aroma and flavor, honeys of orange and eucalyptus achieve higher values as compared to honeys from wildflowers. Therefore, the pollen analysis is a useful analytical tool to certify the botanical origin of the honey and can influence its market value.

Total phenolic contents and antioxidant activity

The results obtained for the total phenolic content and antioxidant activity of the honey samples are shown in Table IV. The total content of phenolic compounds varied from 30.40 to

176.07mg CE/100g honey, respectively for orange honey (numbered 8) and 'bracatinga' honey (numbered 2).

Five samples (numbered 2, 3, 7, 9 and 11) showed total phenolic contents ranging from 75.53 to 176.07mg CE/100g honey. The total phenolic contents of five different types of Yemeni honey and four types of imported honey were evaluated by Al-Mamary *et al.* (2002) and the results ranged from 75.13mg CE/100g honey in Tropical blossom (Marbai-Hadramout) to 246.21mg CE/100g honey in *Acacia ehrenbergina* (Salam-Tehamah). The imported honey samples (Swiss-blossom: 68.59, Iranian: 56.32 and American-orange source: 61.05mg CE/100g honey) had lower total phenolic contents as compared with the Yemeni honeys and also with the honeys numbered 2, 3, 7, 9 and 11 from the Campos Gerais region, except for that of the American-tropical blossom honey, which contained a slightly higher total phenolic contents (79.37mg CE/100g honey).

Samples 2, 3 and 9, which showed enhanced phenolic content, were classified as dark-amber or amber. As indicated in the literature (Kaškonienė *et*

TABLE IV
BOTANICAL ORIGIN, TOTAL PHENOLIC CONTENTS AND ANTIOXIDANT
ACTIVITY FOR SAMPLES OF BEE (*Apis mellifera* L.) HONEY FROM CAMPOS
GERAIS REGION OF BRAZIL

Samples	Botanical origin	Mean \pm SD	
		Total phenolic (mg CE/100g honey)	Antioxidant activity (%)
1	eucalipto (<i>Eucalyptus</i> sp.)	64.37 \pm 3.39	45.11 \pm 4.52
2	bracatinga (<i>Mimosa scabrella</i>)	176.07 \pm 2.53	75.23 \pm 0.76
3	pau-de-malho (<i>Machaerium</i> sp.), yerba mate (<i>Ilex</i> sp.) and aroeira (<i>Lithraea</i> sp.)	104.27 \pm 2.35	56.96 \pm 2.82
4	cipó-uva (<i>Serjania</i> sp.), eucalipto (<i>Eucalyptus</i> sp.), orange (<i>Citrus</i> sp.) and assa-peixe (<i>Vernonia</i> sp.)	50.63 \pm 1.98	37.88 \pm 1.34
5	angico (<i>Piptadenia</i> sp.)	41.72 \pm 1.32	39.22 \pm 0.65
6	eucalipto (<i>Eucalyptus</i> sp.) and laranjinha-do-mato (<i>Zanthoxylum</i> sp.)	50.22 \pm 4.05	36.55 \pm 2.28
7	eucalipto (<i>Eucalyptus</i> sp.)	96.00 \pm 2.86	72.69 \pm 2.63
8	orange (<i>Citrus</i> sp.)	30.40 \pm 0.74	31.66 \pm 1.11
9	yerba mate (<i>Ilex</i> sp.) and aroeira (<i>Lithraea</i> sp.)	118.97 \pm 0.95	74.97 \pm 1.98
10	laranjinha-do-mato (<i>Zanthoxylum</i> sp.), yerba mate (<i>Ilex</i> sp.) and aroeira (<i>Lithraea</i> sp.)	66.10 \pm 4.12	58.63 \pm 1.00
11	aroeira (<i>Lithraea</i> sp.), angico (<i>Piptadenia</i> sp.) and vassoura (<i>Bacharis</i> sp.)	75.53 \pm 1.21	68.67 \pm 2.12
12	angico (<i>Piptadenia</i> sp.) and <i>Myrcia</i> type	43.13 \pm 0.95	45.98 \pm 1.99
13	laranjinha-do-mato (<i>Zanthoxylum</i> sp.), yerba mate (<i>Ilex</i> sp.) and <i>Myrcia</i> type	47.55 \pm 1.41	44.51 \pm 0.84

al., 2009), this result suggests a relation between the intensity of honey color and the total content of phenolic compounds. However, one sample (numbered 7) showed a high content of phenolic compounds and was determined as extra-light amber.

For the DPPH radical-scavenging activity, monofloral (2 and 7) and polyfloral (9 and 11) samples showed improved antioxidant capacities as compared to other honeys studied. However, these values were lower than that those observed for the 200mg·l⁻¹ ethanol solution of α -tocopherol, which indicated an antioxidant capacity of 91.51%.

The phenolic contents of honey and consequently its antioxidant capacity depend on the floral sources used to collect honey, and its predominance is dependent upon seasonal and environmental factors (Al-Mamary *et al.*, 2002).

Some compounds such as flavonoids, phenolic acids, and phenolic diterpenes have antioxidative effects. Studies carried out by Hodnick *et al.* (1988) revealed that the flavonoids with the most hydroxyl groups were most eas-

ily oxidized. The differences in activities of antioxidants depend on structural dissimilarities, primarily on the degree of hydroxylation and methylation of the compounds. Moreover, the data reported by Gazzani *et al.* (1998) demonstrate that some phenolic compounds may react as antioxidants faster than others under the same conditions. Furthermore, the presence of other constituents than the phenolic compounds, such as vitamins C and E, carotenoids, peptides, organic acids and enzymes may influence the total antioxidant activity of honey (Al-Mamary *et al.*, 2002).

The high correlation coefficient ($r=0.8379$) that was calculated using the total phenolic content and the antioxidant activity data indicates that phenolics are one of the main components related to the antiradical activity of honey. This statistically significant correlation coincides with the findings of other authors (Turkmen *et al.*, 2006; Baltrusaityte *et al.*, 2007; Burratti *et al.*, 2007; Lachman *et al.*, 2010; Silici *et al.*, 2010) who also found a strong relationship between antiradical capacity and

total phenolic content of honey.

Honey can be used as a preservative in food, as it prevents oxidation reactions such as lipid oxidation in meat and enzymatic browning of fruits and vegetables (Oszmianski and Lee, 1990; Chen *et al.*, 2000; Mckibben and Engeseth, 2002). Moreover, the presence of antioxidant compounds plays an important role in disease prevention (Berg *et al.*, 1999), as is the case in coronary heart disease, arthritis, diabetes, neurodegenerative diseases (e.g., Parkinson's disease), cancer and ethanol intoxication (Benzie and Strain, 1996; Russo *et al.*, 2002).

In conclusion, most of the honey samples analyzed showed physicochemical properties in accordance to the Brazilian and international legislation. Regarding the botanical origin, all samples were established as floral honeys. However, a lack of correspondence between the botanical origin described by beekeepers and melissopalynological data were verified. A correlation between total phenolic content and antioxidant activity was observed for all the samples studied.

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REFERENCES

- Al-Mamary M, Al-Meeri A, Al-Habouri M (2002) Antioxidant activities and total phenolics of different types of honey. *Nutr. Res.* 22: 1041-1047.
- AOAC (2000) *Methods of analysis of AOAC International*. Association of Official Analytical Chemists. Gaithersburg, MD, USA.
- Baltrusaityte V, Venskutonis PR, Ceksteryte V (2007) Radical scavenging activity of differential floral origin honey and beebread phenolic extracts. *Food Chem.* 101: 502-514.
- Barth OM (1989) *O Pólen no Mel Brasileiro*. Luxor. Rio de Janeiro, Brazil. 150 pp.
- Barth OM (2004) Melissopalynology in Brazil: a review of pollen analyses of honeys, própolis and pollen loads of bees. *Sci. Agr.* 61: 342-350.
- Barth OM (2005) Análise polínica de mel: avaliação de dados e seu significado. *Mensagem Doce* 81: 2-6.
- Barth OM, Maiorina C, Benatti APT, Bastos DHM (2005) Determinação de parâmetros físico-químicos e da origem botânica de méis indicados monoflorais do sudeste do Brasil. *Cienc. Tecnol. Alim.* 25: 229-233.
- Bath PK, Sing NA (1999) A comparison between *Helianthus annuus* and *Eucalyptus lanceolatus* honey. *Food Chem.* 67: 389-397.
- Benzie IFFE, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": the FRAP assay. *Anal. Biochem.* 239: 70-76.
- Berg RVD, Haenen GRMM, Berg HVD, Bast A (1999) Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem.* 66: 511-517.
- Bianchi EM (1981) *La Miel, Características y Composición - Análisis y Adulteraciones*, UNSE-CEDIA. Santiago del Estero, Argentina. 158 pp.
- Bogdanov S, Martin P, Lullmann C (1997) Harmonised methods of the European Honey Commission. *Apidologie* 28(Extra Issue): 1-59.
- Borsato DM, Vargas T, Koop L, Faraço PV, Almeida MMde (2010) Physicochemical quality control of bee honeys from Campos

- Geraias region of Paraná - Brazil. *B. CEPPA* 28: 205-212.
- Brasil (2000) *Instrução Normativa Número 11, de 20 de Outubro de 2000. Regulamento Técnico de Identidade e Qualidade do Mel*. Ministério da Agricultura, Pecuária e Abastecimento. Brasília, Brazil. Diário Oficial da União (23/10/2000) Seção I. pp. 16-17.
- Buratti S, Benedetti S, Cosio MS (2007) Evaluation of the antioxidant power of honey, propolis and royal jelly by amperometric flow injection analysis. *Talanta* 71: 1387-1392.
- Chen CN, Wu CL, Shy HS, Lin JK (2003) Cytotoxic prenylflavonones from Taiwanese propolis. *J. Nat. Prod.* 66: 503-506.
- Chen L, Mehta A, Berenbaum M, Zanger AR, Engeseth NJ (2000) Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. *J. Agric. Food Chem.* 48: 4997-5000.
- Codex (2001) *Codex Standard 12-1981: Revised Codex Standard for Honey, Standards and Standard Methods*. Rev 1 (1987), Rev 2 (2001). Codex Alimentarius Commission. Rome, Italy.
- Cook NC, Sammon S (1996) Flavonoids - Chemistry, metabolism, cardioprotective effects, and dietary sources. *J. Nutr. Biochem.* 7: 66-76.
- Crane E (1983) *O Livro do Mel*. Nobel. São Paulo, Brazil. 226 pp.
- Ferreres F, Tomás-Barberán FA, Gil MI, Tomás-Lorente F (1991) An HPLC technique for flavonoid analysis in honey. *J. Sci. Food Agric.* 56: 49-56.
- Gazzani G, Papetti A, Daglia M, Berte F, Gregotti C (1998) Protective activity of water soluble components of some common diet vegetables on rat liver microsomes and the effect of thermal treatment. *J. Agric. Food Chem.* 46: 4123-4127.
- Gheldof N, Engeseth NJ (2002) Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of vitro lipoprotein oxidation in human serum samples. *J. Agric. Food Chem.* 50: 3050-3055.
- Giorgi A, Madeo M, Baumgartner J, Lozzia GC (2011) The relationships between phenolic content, pollen diversity, physicochemical information and radical scavenging activity in honey. *Molecules* 16: 336-347.
- Horn H (1997) Méis brasileiros: resultados de análises físico-químicas e palinológicas. *Mensagem Doce* 40: 10-16.
- Hodnick WF, Milosavljevic EB, Nelson JH, Pardini RS (1988) Electrochemistry of flavonoids. *Biochem. Pharmacol.* 37: 2607-2611.
- Hussein SZ, Yusoff KM, Makpol S, Yusoff YAM (2011) Antioxidant capacities and total phenolic contents increase with gamma irradiation in two types of malaysian honey. *Molecules* 16: 6378-6395.
- Kaškonienė V, Maruška A, Kornýšova O, Charczun N, Ligor M, Buszewski B (2009) Quantitative and qualitative determination of phenolic compounds in honey. *Chem. Technol.* 52: 74-80.
- Kirkwood KC, Mitchell TJ, Smith D (1960) Examination of the occurrence of honeydew in honey. *Analyst* 85: 412-416.
- Kirkwood KC, Mitchell TJ, Smith D (1961) Examination of the occurrence of honeydew in honey. Part II. *Analyst* 86: 164-165.
- Kishore RK, Halim AS, Syazana MS, Sirajudeen KN (2011) Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. *Nutr. Res.* 31: 322-325.
- Komatsu SS (1996) *Caracterização Físico-Química de Méis de Apis mellifera L. 1758 (Hymenoptera: Apidae) de Diferentes Municípios do Estado de São Paulo*. Tese. Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Brazil. 90 pp.
- Lachman J, Orsák M, Hejtmánková AH, Kovářova E (2010) Evaluation of antioxidant activity and total phenolics of selected Czech honeys. *LWT-Food Sci. Technol.* 43: 52-58.
- Liberato MdaCTC, Morais SMde, Siqueira SMC, Menezes JESde, Ramos DN, Machado LKA, Magalhães IL (2011) Phenolic content and antioxidant and anti-acetylcholinesterase properties of honeys from different floral origins. *J. Med. Food* 14: 658-663.
- Louveaux J, Maurizio A, Vorwohl G (1970) Methods of melissopalynology. *Bee World* 5: 125-138.
- Mckibben J, Engeseth NJ (2002) Honey as a protective agent against lipid oxidation in ground turkey. *J. Agric. Food Chem.* 50: 592-595.
- Melhem TS, Cruz-Barros MAVda, Corrêa AMda S, Makino-Watanabe H, Silvestre-Capelato, MSF, Esteves VLG (2003) *Variabilidade Polínica em Plantas de Campos do Jordão (São Paulo, Brasil)*. Boletim do Instituto de Botânica. N° 16. São Paulo, Brasil. 104 pp.
- Moreira RFA, De Maria CAB (2001) Glicídios no mel. *Quim. Nova* 24: 516-525.
- Nagai T, Inoue R, Kanamori N, Suzuki N, Nagashima T (2006) Characterization of honey from different floral sources. Its functional properties and effects of honey species on storage of meat. *Food Chem.* 97: 256-262.
- Nair PKK (1960) A modification in the method of pollen preparation. *J. Sci. Indust.* 19C: 26-27.
- National Honey Board (2011) *Properties of Honey*. www.nhb.org/properties/index.html
- Oszmianski J, Lee CY (1990) Inhibition of polyphenol oxidase activity and browning by honey. *J. Agric. Food Chem.* 38: 1892-1895.
- Russo A, Longo RE, Vanella A (2002) Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. *Fitoterapia* 73: S21-S29.
- Santos KS, Malaspina O, Palma MS (2003) Cinética da diástase em méis de diferentes origens florais. Um novo protocolo experimental. *Mensagem Doce* 70: 2-4.
- Silici S, Sagdic O, Ekici L (2010) Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys. *Food Chem.* 121:238-243.
- Singleton VL, Orthofer R, Lamuela RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth. Enzymol.* 299: 152-178.
- Takeda IJM, Farago PV, Souza MKFde, Gelinski VV (2000) Catálogo polínico do Parque Estadual de Vila Velha, Paraná. 1ª Parte. *Biol. Health Sci. (UEPG)* 6: 61-73.
- Takeda IJM, Farago PV, Souza MKFde, Gelinski VV (2001) Catálogo polínico do Parque Estadual de Vila Velha, Paraná. 2ª Parte. *Biol. Health Sci. (UEPG)* 7: 7-18.
- Takeda IJM, Farago PV, Souza MKFde, Gelinski VV (2002a) Catálogo polínico do Parque Estadual de Vila Velha, Paraná. 3ª Parte. *Arq. Cs. Saúde Unipar* 6: 61-66.
- Takeda IJM, Farago PV, Souza MKFde, Gelinski VV (2002b) Catálogo polínico do Parque Estadual de Vila Velha, Paraná. 4ª Parte. *Arq. Cs. Saúde Unipar* 6: 97-100.
- Tosi E, Martinet R, Ortega M, Lucero H, Ré E (2008) Honey diastase activity modified by heating. *Food Chem.* 106: 883-887.
- Turkmen N, Sari F, Poyrazoglu ES, Velioglu YS (2006) Effects of prolonged heating on antioxidant activity and colour of honey. *Food Chem.* 95: 653-657.
- Vargas T (2006) *Caracterização da Qualidade do Mel Produzido nos Campos Gerais*. Tese. Universidade Estadual de Ponta Grossa. Brazil. 150 pp.
- Von Der Ohe W, Persano-Oddo L, Piana ML (2004) Harmonized methods of melissopalynology. *Apidologie* 35: S18-S25.
- White JWJr (1975) Physical Characteristics of Honey. In Crane E (Ed.) *Honey: A Comprehensive Survey*. Heinemann. London, UK. pp. 157-206.
- Yen G, Wu J (1999) Antioxidant and radical scavenging properties of extracts from Ganoderma tsugae. *Food Chem.* 65: 375-379.
- Zumla A, Lulat A (1989) Honey: a remedy rediscovered. *J. Roy. Soc. Med.* 82: 384-385.